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Effects of Fitness and Age on the Response to Vagotonic Atropine

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Abstract

Previous work indicates compromised cardiac vagal control plays a prominent role in reducing arterial baroreflex gain with age, however older fit individuals display cardiovagal baroreflex responses similar to young individuals. The purpose of this study was to test the hypothesis that chronic aerobic exercise mitigates against age-related declines in cardiac parasympathetic receptor function. In forty-four young and old (fit and unfit) individuals, we used the parasympathomimetic responses to low doses of atropine to probe cardiac cholinergic receptor responses. Data was collected before and after eight doses of atropine sulfate from 0.4 to 7.2 ug/kg. Chronotropic responses were assessed from average RR intervals and heart rate variabilities were derived in time and frequency domains. All subjects exhibited bradycardia with at least one dose of atropine and peak bradycardia occurred at a similar dose in each group. However, changes in heart rate variability did not consistently track the chronotropic responses within subjects (r-square from 0.90 down to 0). As expected, basal RR interval was longer in the fit groups and was unaffected by age. However, the degree of RR interval lengthening with parasympathomimetic atropine was unaffected by physical fitness and was significantly less in all older subjects. These data indicate there are certain prepotent age-related declines in the cardiac parasympathetic system that cannot be prevented by regular physical activity.

Keywords

atropine; vagal; heart rate variability; muscarinic receptor

Our previous work indicates a decline in cardiac parasympathetic neural control compromises baroreflex gain with age (Kaushal et al., 2002) and this decline can be offset by regular physical activity (Hunt et al., 2001). This suggests a reduced cardiac vagal control accompanying physiologic aging may result essentially from progressive deconditioning (Goldsmith et al., 1997). Greater resting cardiac vagal tone is a distinguishing characteristic of physically fit individuals (Ekblom et al., 1973; Frick et al., 1967; Shi et al., 1995; Smith et al., 1989) and a vagally mediated resting bradycardia is observed in younger as well as older humans after exercise training (Stratton et al., 1994). Moreover, our prior work shows that older fit individuals can display baroreflex-mediated cardiac vagal control similar to young individuals (Hunt et al., 2001). In these individuals, baroreflex responses are maintained despite age-related

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arterial stiffening similar to sedentary individuals, indicating that exercise training may be a powerful stimulus to prevent vagal declines with age (Hunt et al., 2001).

Atropine sulfate is routinely used to probe the cardiac parasympathetic system. For example, the chronotropic response to high, fully parasympatholytic doses of atropine has been used to quantify resting cardiac vagal tone (Robinson et al., 1966). However, very low doses are parasympathomimetic reducing heart rate (Averill et al., 1959; McGuigan, 1921; Morton et al., 1958) and increasing variability (Raczowska et al., 1983). The responses to vagotonic atropine may provide insight to mechanisms influencing resting cardiac vagal tone. The preponderance of evidence suggests that low dose atropine preferentially blocks presynaptic cholinergic autoreceptors in the heart (Brodde et al., 2001; Epstein et al., 1990; Hellgren et al., 2000; Oberhauser et al., 2001), increasing the release of acetylcholine from parasympathetic nerves. If receptor function is decreased with age or is enhanced by exercise training, the parasympathomimetic response would change accordingly. The present cross-sectional study sought to determine influences of aging and chronic aerobic exercise on these responses. We used sequential, low doses of atropine to determine maximal parasympathomimetic effect and compared responses in young and older, fit and unfit individuals. We hypothesized chronic aerobic exercise would augment responses to vagotonic atropine and mitigate against age-related declines in the response.

METHODS

Subjects

Forty-four healthy volunteers (23 male and 21 female) participated in the study. All volunteers were free from cardiovascular diseases as determined by health history questionnaires, fasting blood chemistries, and resting and maximal exercise electrocardiograms. Volunteers were excluded if they presented a history of any cardiovascular disease, depression, neurological disorders, diabetes, and obesity or if they were taking any cardioactive medications. All volunteers were nonsmokers and normotensive. Volunteers were instructed to refrain from consuming caffeine and alcohol and from performing any vigorous activity 24 hours prior to testing.

Subjects were classified into 4 groups based on age and habitual physical activity levels (Table 1). Young subjects were age 21–30 and older subjects were age 55–75. Unfit subjects did not regularly participate in aerobic exercise and were excluded if they performed aerobic exercise for at least 30 minutes, 3 times per week. Fit subjects performed aerobic exercise for a minimum of 30 minutes, 5 times per week and had maintained their current training level for at least 1 year. In addition, each subject underwent maximal oxygen consumption ($\text{VO}_{2\text{max}}$) determination during graded treadmill exercise to document that all fit subjects had maximal aerobic powers above the 90th percentile for individuals of their age and gender (American College of Sports Medicine, 2000). Standard criteria were applied to ensure all subjects achieved maximal aerobic consumption (i.e., respiratory exchange ratio ≥ 1.10 , heart rate at least 85% of age-predicted, plateau in oxygen consumption with increasing workload, and a maximal rating of perceived exertion).

To verify activity levels, each subject completed a Stanford 7-Day Physical Activity Recall Questionnaire (Table 1) (Richardson et al., 2001). This is a well known validated instrument which assesses physical activity performed at different intensities over the previous seven days. The questionnaire divides physical activity into 4 categories; very hard (10 METs), hard (6 METs), moderate (4 METs) and light activity (1.5 METs).

This protocol was approved by the Institutional Review Board of the Hebrew Rehabilitation Center for Aged and conformed with The Declaration of Helsinki. Each subject was thoroughly acquainted with all aspects of the experiment prior to obtaining informed consent.

Experimental protocol

Each subject reported to the laboratory at least two days after the $\text{VO}_{2\text{max}}$ test, between 7:00 am and 11:00 am following a 12-hour overnight fast. Following instrumentation and insertion of an antecubital venous catheter for drug infusion, subjects rested in the supine position for at least 10 min prior to data collection. During all data collection periods, subjects controlled their breathing frequency at 15 breaths/minute (0.25 Hz) in response to an auditory cue. Each data collection period consisted of five minutes from which the last three minutes was extracted for analysis. Data was collected at baseline and after eight bolus doses of atropine sulfate (0.4, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.4 $\mu\text{g/kg}$; cumulative doses of 0.4, 0.8, 1.4, 2.2, 3.2, 4.4, 5.8, and 7.2 $\mu\text{g/kg}$). After each injection, at least two minutes was allotted for development of full drug effect prior to data acquisition. If subjects demonstrated a significant tachycardia (heart rate ~ 10 beats/min above baseline) before the eighth dose, atropine administration was stopped. Atropine has a half-life of approximately 4 hours; therefore, the duration of our protocol was sufficiently short that effects were sustained throughout all measurement periods.

Measurements

Arterial blood pressure was recorded every minute from the right arm by an automated brachial cuff (Dinamap, Critikon). RR interval was determined from lead II of an electrocardiogram. Respiration was determined by a respiratory transducer bands placed around the midchest.

Data Analysis

Data were continuously digitized and stored on a personal computer at a sampling rate of 500 Hz (DI-700 & WinDaq, DATAQ Instruments, OH). Beat-by-beat RR intervals were determined using peak detection algorithms (Mathworks Inc, Natick, MA) and manually inspected to confirm data quality. Average blood pressures and RR intervals were determined from three minutes of paced breathing for each atropine dose. Chronotropic responses were assessed from RR interval because it most linearly reflects changes in parasympathetic stimulation (Koizumi et al., 1985; Parker et al., 1984). Heart rates were also derived to confirm that the peak chronotropic responses occurred at the same atropine dose regardless of chronotropic measure. RR interval standard deviation and the root of the mean square of successive differences in RR intervals were taken as representative time domain measures. The power spectra of 4 Hz re-sampled RR interval time series were calculated via fast Fourier transforms based on Welch's periodogram algorithm. Total spectral power below 0.5 Hz (i.e., total power) and respiratory power at 0.25 Hz (i.e., respiratory sinus arrhythmia) were derived as representative frequency domain measures. These measures of heart rate variability have been proposed to be standard and valid measures of cardiac vagal tone (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

Statistics

Data were compared across four groups using a two-way ANOVA to examine effects of fitness and age. Possible gender differences and interactions were tested, but none were found (lowest $P=0.16$, for dose-6 RR interval, gender $\times$ fitness). Therefore, only results from age and fitness and their interactions are presented. To examine the relationships between parasympathetically mediated cardiac chronotropy and RR interval variability measures, linear regressions were performed between the mean RR interval and each HRV index across all atropine doses within

each subject. All values are presented as mean $\pm$ SE and statistical significance was accepted at $P < 0.05$.

RESULTS

The protocol dictated atropine dosing was stopped if a tachycardia of 10 or more beats/min above baseline occurred before the eighth dose. As a result, two subjects had six doses, 22 subjects had seven doses, and 20 subjects had eight doses of atropine. Figure 1 depicts the average changes in RR interval across atropine doses for each group. All subjects exhibited bradycardia in response to at least one dose of atropine. The dose at which peak bradycardia occurred was not different across groups and developed with the fourth dose, or 2.2 ug/kg of atropine in two-thirds of the subjects. On average, arterial pressures were unchanged by lower drug infusion levels, but increased slightly at the highest doses (i.e., At dose seven, 5.8 ug/kg atropine, systolic pressure increased 3.7 ± 1.3 mmHg and diastolic pressure increased 2.8 ± 0.9 mmHg, $P < 0.05$). These responses did not differ between groups.

Basal RR interval was longer in the fit groups and was unaffected by age (Figure 2). Likewise, the peak RR interval with parasympathomimetic atropine was longer in the fit groups and was unaffected by age. However, the magnitude of RR interval lengthening with parasympathomimetic atropine was unaffected by physical fitness and was significantly less in both older groups compared with the young. Thus, exercise training resulted in a resting bradycardia, but had no effect on the response to parasympathomimetic atropine and did not prevent the age-related decline in the response. These differences remained when heart rate was used to assess bradycardiac responses.

Basal heart rate variabilities in both time and frequency domains were significantly reduced with age ($P < 0.05$ for all). However, changes in the variabilities did not consistently track chronotropic responses to parasympathomimetic and parasympatholytic atropine. Figure 3 shows data from two subjects who exemplify the range of patterns demonstrated by the heart rate variability indices across doses of parasympathomimetic and parasympatholytic atropine. As a result of this inconsistency, the relationship between variabilities and mean RR interval across doses of atropine demonstrated wide variations for within subject correlations, for standard deviation from -0.35 to 0.93 , for root means square of successive differences from -0.79 to 0.95 , for respiratory sinus arrhythmia from -0.80 to 0.90 , and for total spectral power from -0.23 to 0.93 . The dose of atropine eliciting the largest bradycardia was infrequently the dose of atropine eliciting the largest increase in variability. In fact, the peak increase in variabilities only occurred at the peak vagotonic dose for RR interval less than one third of the time. In many cases at the peak vagotonic dose for RR interval, variability was reduced below basal levels. Figure 4 shows the responses averaged across subjects within each group.

DISCUSSION

We sought to explore interactions between exercise- and age-related effects on the cardiac parasympathetic system. We hypothesized chronic aerobic exercise augments responses to vagotonic atropine and mitigates against age-related declines in cholinergic receptor function. However, we were surprised to find that fitness level does not impact responses to low doses of atropine. Although our data support previous findings that both young and older trained individuals demonstrate a resting bradycardia (Spina et al., 1998; Stratton et al., 1994), they suggest it does not relate to the responses to vagotonic atropine. Moreover, our data indicate prepotent age-related declines in cardiac cholinergic receptor function cannot be prevented by regular physical activity, even in highly trained endurance athletes. Considering prior data demonstrating maintained baroreflex control of cardiac vagal effects in older fit individuals,

it appears vagotonic atropine exerts its effects at a site in the reflex pathway which is unaffected by exercise training.

The case has been made by some that the mechanism for atropine's vagomimetic effects are of central neural origin (Raczkowska et al., 1983). For example, cardiac vagal efferent activity in anesthetized dogs increases after atropine doses of approximately 4.86 micrograms/kg and higher (Katona et al., 1977). The response in humans with transplanted hearts has also been cited as support of a central neural mechanism. The donor sinus node does not respond to any dose of atropine, either vagotonic or vagolytic, whereas the native innervated sinus node demonstrates bradycardia at atropine doses less than 2 microgram/kg and tachycardia at higher doses (Epstein et al., 1990). However, anesthetized animals may not provide the best model of human autonomic responses and data from human transplanted hearts only indicate vagal innervation is required to observe the chronotropic responses to atropine.

There is strong evidence to indicate that the bradycardia with low dose atropine originates from peripheral receptor effects. For example, intravenous dosages that produce bradycardia result in no measurable atropine in human cerebral spinal fluid (Virtanen et al., 1982). Moreover, low doses of an atropine derivative that cannot cross the blood brain barrier also induce bradycardia (Kottmeier et al., 1968), indicating effects of low dose atropine may develop from blockade of peripheral muscarinic receptors. However, bradycardia cannot be generated by antagonism of post-synaptic cardiac muscarinic receptors, but only by antagonism of pre-synaptic M1 muscarinic autoreceptors. Selective stimulation of M1 receptors increases heart rate (Shannon et al., 1994), and selective blockade decreases heart rate (Jakubetz et al., 2000; Pitschner et al., 1988; Wellstein et al., 1988). Since there are no M1 receptors on the myocardium itself, agonist or antagonist mediated changes in heart rate must derive from altered acetylcholine release from cardiac parasympathetic nerves. It seems likely low dose atropine generates vagomimetic effects similarly, by blocking presynaptic muscarinic autoreceptors, enhancing acetylcholine release, and causing negative chronotropic effects through activation of post-synaptic M2 receptors. There is direct evidence for this: pirenzepine, a hydrophilic, selective M1 antagonist abolishes the bradycardia with low dose atropine but does not affect the tachycardia with high dose atropine (Wellstein et al., 1988). Thus, the preponderance of data suggests low dose atropine generates a bradycardia via blockade of pre-synaptic muscarinic autoreceptors.

Considering the above, lesser heart rate slowing in older adults in response to parasympathomimetic atropine reflects impairments in pre-synaptic and/or post-synaptic muscarinic receptor function. Previous research demonstrated both atropine and pirenzepine result in lesser bradycardia in older subjects (Poller et al., 1997), however response to either drugs is the sum of pre-synaptic receptor blockade and post-synaptic receptor stimulation. There are no data specifically examining the effect of age on pre-synaptic M1 receptors, although lesser release of acetylcholine from atria in older humans has been interpreted as reflecting *up-regulation* of pre-synaptic autoreceptors (Oberhauser et al., 2001). If this were the case in our subjects, we would have observed greater, not lesser bradycardia in older subjects with low dose atropine. The only direct evidence of cardiac muscarinic receptor change with age indicates lesser density and lower affinity of post-synaptic M2 receptors in adult humans (Brodde et al., 1998) and lesser mRNA and protein levels of M2 cholinergic receptors in older rats (Lo et al., 2001). However, some animal data suggest acetylcholinesterase activity is reduced with age (Kennedy et al., 1990) which would effectively increase the relative amounts of acetylcholine reaching post-synaptic receptors. If this applies to humans, it suggests aging causes profound declines in cholinergic receptor function.

Regardless of pre- or post- synaptic deficit, our data provide support that human aging markedly reduces cardiac muscarinic receptor function regardless of physical fitness. We reasoned that

the resting, training-related bradycardia in both young and older fit individuals would relate to greater slowing of heart rate with vagotonic atropine. However, we did not find this; in fact, even though our older fit subjects tended to be slightly younger, their bradycardiac response was no different from their unfit peers. This suggests that, regardless of age, increased parasympathetic muscarinic receptor function does not contribute to the training bradycardia. However, resting heart rate reflects both intrinsic rate and autonomic influences and data suggest that the resting bradycardia in trained individuals derives in part from a lower intrinsic rate (Katona et al., 1982) but with a parasympathetic predominance (Smith et al., 1989). Thus, decreases in intrinsic heart rate (Randall et al., 2003; Scott et al., 2004) may play a more vital role in the resting bradycardia that results from regular aerobic conditioning. In addition, there may be a ceiling for maximal vagal activity and vagal effects on the heart may be greatest in young adult humans and may not be able to be enhanced. Both cross-sectional (Smith et al., 1988) and longitudinal data (Kingwell et al., 1992) suggest that cardiac vagal baroreflex control is not enhanced by endurance training in young individuals. In contrast, our prior work showed that baroreflex gain is preserved in older active individuals and this was achieved primarily through enhanced neural control (Hunt et al., 2001). Our current data suggest that cholinergic receptor declines are a primary, age-associated change that are unaffected by exercise training. This type of receptor decline may not be surprising since it has also been shown that reduced cardiac beta-adrenergic responsiveness is also a primary age-associated decline that is unaffected by physical activity (Stratton et al., 1992). Considering our prior and current results, exercise training may result in central neural adaptations that are sufficient to overcome age-related cardiac autonomic receptor deficits.

We derived time and frequency domain estimates of heart rate variability widely accepted as valid indices of cardiac vagal tone (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). We presumed these indices would provide additional insight to the vagotonic effects of low dose atropine. Although absolute RR interval is the best reflection of vagal effects (Carlsten et al., 1957; Koizumi et al., 1985; Parker et al., 1984; Stuesse et al., 1981), there exists a conventional wisdom that heart rate variability indices provide useful metrics for cardiac vagal effects. However, we found that none of the variability indices we derived tracked the progressive, cholinergically mediated, chronotropic effects of atropine. Respiratory pattern is unlikely the culprit for our finding. All subjects adequately maintained eupneic breathing at a frequency of 0.25 Hz for measurement of variabilities. Although our finding may be analogous to data demonstrating the relationship between heart rate variability and parasympathetic effect is best described by a nonlinear function with marked interindividual variation (Goldberger et al., 2001), it underscores the latter aspect. If variability reflects sinusoidal vagal modulation of heart rate and not vagal tone, our data show this modulation can change in an unpredictable manner in some individuals and provide a cautionary note on heart rate variabilities.

One limitation of this study is that we did not obtain the heart rate dose response curve up to a fully parasympatholytic dose of atropine. We used successive incremental doses of atropine to induce a parasympathomimetic effect and then a partial parasympatholytic effect. Reaching the plateau phase of the heart rate response would have allowed calculation of parasympathetic tone in the subject groups and provided additional information on vagal control. However, to use this full atropine blockade data for inferences on vagal tone, we would have needed to block cardiac sympathetic effects first and this would have obscured the responses to the low doses of atropine. Thus, this approach would not only require a different study design, but would provide no information on possible cholinergic receptor effects of aging and physical activity.

Our results indicate chronic aerobic exercise does not augment responses to vagotonic atropine. From the available evidence, this suggests bradycardia in both young and older trained

individuals is not a receptor-mediated phenomenon. Moreover, our data suggests exercise training does not mitigate against age-related declines in cardiac muscarinic receptor function. Therefore maintained baroreflex gain in older endurance athletes must derive from vagal neural adaptations which overcome declines in cardiac muscarinic receptor function that may be a fundamental characteristic of human aging.

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Figure 1.

Changes in RR interval with progressive low dose atropine in young and older, fit and unfit individuals. Atropine dose 8 (7.2 ug/kg) appears inconsistent in its effect because only 20 of 44 subjects received this dose. Values are mean $\pm$ S.E.M.

Figure 2.

Average basal and peak RR intervals and peak changes in RR interval with low dose atropine in young and older, fit and unfit individuals. Values are mean $\pm$ S.E.M

Figure 3.

Examples of the responses to low dose atropine. One subject had a consistent pattern in both of RR interval and heart rate variabilities and the other did not.

Figure 4.

Average responses of heart rate variabilities to progressive low dose atropine in young and older, fit and unfit individuals. Values are mean $\pm$ S.E.M.

Table 1

Subject Characteristics

n (females)	Young Fit 11 (6)	Young Unfit 11 (5)	Old Fit 11 (4)	Old Unfit 11 (6)
age, yrs	24.0±0.8	24.6±0.8	59.0±1.2	66.1±2.0
bmi, kg/m ²	24.1±0.6	22.3±1.0	22.7±0.8	24.2±0.9
weight, kg	73.1±2.3	69.9±5.0	65.5±4.6	68.7±3.2
systolic pressure, mmHg	112±6	108±3	123±5	110±3
diastolic pressure, mmHg	66±5	61±2	76±3	70±2
maximum VO ₂ , ml/kg/min	55.5±2.3	43.0±1.4	51.4±2.8	27.8±1.0
length of training, years	4.7±1.3		21.5±4.2	
Weekly activity, minutes				
moderate	193±53	305±44	285±95	294±53
hard	19±12	36±15	141±52	63±32
very hard	262±57	57±22	289±57	39±28

All values are mean±S.E.M.